A DISEASE OF FOWLS IN PALESTINE CHARACTERISED BY LEUCOCYTE INCLUSIONS

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Mr. D. Ury, the director of an experimental poultry farm at Ben-Shemen, Palestine, called my attention to a disease of fowls on his farm which, although in its later stages it resembled spirochaetosis, was not amenable to treatment with atoxyl or neo-salvarsan. The disease is of considerable economic importance, as it attacks Rhode Island and Leghorns and hybrids of the above varieties with native fowls. Native fowls were not observed to be attacked. The first symptoms to be observed were depression and a refusal to take food ; later a tendency to stand still, and fever up to 110° F. ; finally the infected bird was unable to stand, diarrhoea with greenish stools developed and death took place from seven to fourteen days after the commencement of the first symptoms.

Examination of the blood revealed chromatic inclusions in the protoplasm of the leucocytes. The inclusions were of the following varieties :

(1) Minute granules of chromatin surrounded by a vacuole. The protoplasm of cells containing even a few of these forms was often markedly vacuolated.

(2) Small regular rings of chromatin.

(3) Spherical solid masses of chromatin.

(4) Irregular bacilliform masses of chromatin.

(5) Clusters of minute granules of chromatin not lying in vacuoles.

The above kinds of inclusions were also noted inside the nuclei of infected cells.

The normal polymorphs of fowls contain three types of granules.

(1) Spherical granules staining pale red with Romanowsky stains.

(2) Elongated fusiform granules usually staining like eosinophil granules with Romanowsky.

(3) Spherical granules staining deep blue with Romanowsky stains.

From the above types of granules the chromatic inclusions were readily distinguished, being stained with Giemsa like the nuclei of malaria parasites but more brilliantly.

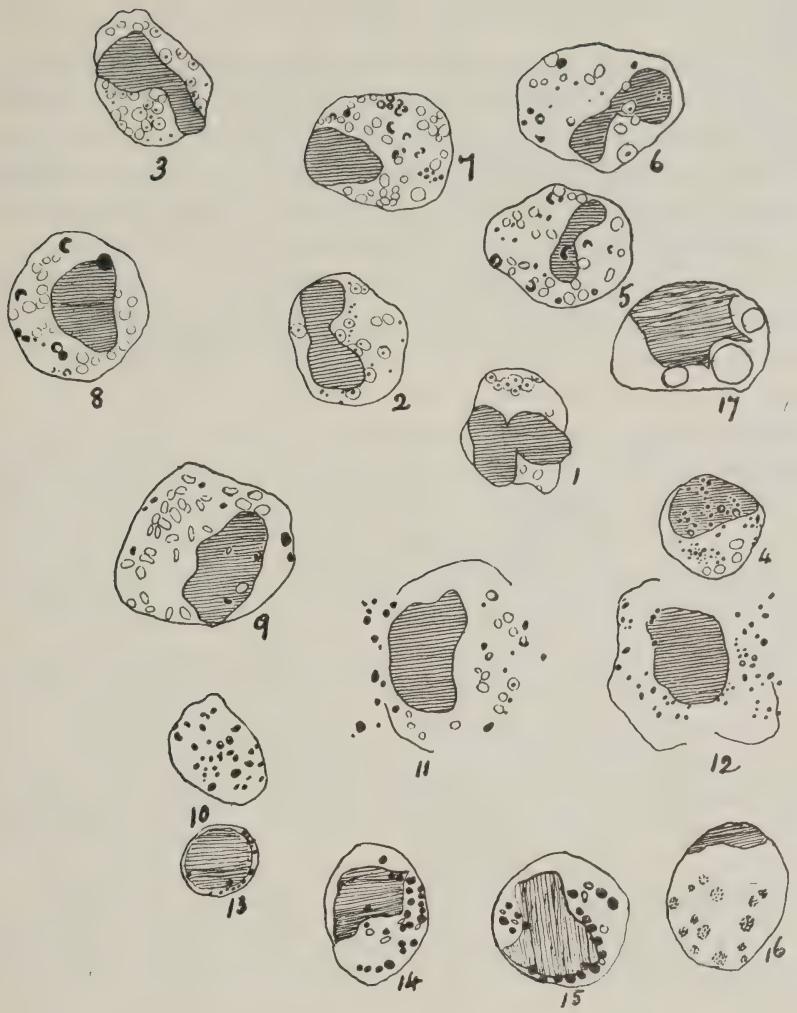
The number of inclusions in a leucocyte varied from one or two to very many ; in some instances the inclusions filled almost the whole cytoplasm of the leucocyte and appeared to escape into the general circulation by bursting the infected cell.

In addition to the inclusion in the leucocytes, small masses of protoplasm containing chromatic rings and solid spheres of chromatin were found in blood smears ; these masses are probably fragments of the cytoplasm of infected leucocytes.

All varieties of leucocytes except eosinophils and mast-cells contained the above-described inclusions, as many as 18 per cent. of the total leucocytes being infected. The nuclei of highly infected cells particularly of lymphocytes, tended to become degenerated and stained feebly with Giemsa in marked contrast to the brilliant staining of the inclusions. In highly infected lymphocytes the nucleus disappeared almost entirely and the cell stuffed with chromatic inclusions had a superficial resemblance to a Koch's blue body ; this form was found particularly in tissue smears. All stages between a slightly infected lymphocyte and the forms resembling Koch's blue bodies were found in tissue smears. A considerable number of the erythrocytes showed basophil staining of the protoplasm and rarely stippling resembling large Schüffner's dots.

Post-mortem the most striking changes were found in the liver and kidneys. The liver was enlarged and soft and studded with white patches ; on section, this organ showed fatty degeneration and infiltration ; the white patches were parts where fatty degeneration was most marked. In the kidneys, patches of necrosis were found.

Smears of the liver, lung, kidneys and spleen showed numerous leucocytes containing inclusions. Auto-erythro-phagocytosis, a phenomenon noted by Levaditi (1914) and by Macfie and Johnston



1 to 3.—Leucocyte with vacuoles some of which contain minute chromatic granules.

4 to 9.—Leucocytes with vacuoles and various forms of inclusions some of them apparently in the nucleus.

10.—Protoplasmic mass containing chromatic inclusions. From a lung smear.

11 to 12.—Leucocytes from which chromatic inclusions are escaping. Figure 11 from a case of *Leukaemia gallinarum.*

13.—An infected Lymphocyte.

14 to 15.—Leucocytes containing solid chromatic inclusions.

16.—A Leucocyte containing clumps of chromatic granules.

17.—An endothelial cell with three fragments of phagocyted erythrocytes.

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(1914) in spirochaetosis of fowls was observed in smears of the organs.

The above described leucocytic inclusions were constantly found in this disease and it therefore appears that they are either casually related to the disease or are the effect of the disease on the leucocytes.

Since the disease was not observed in native fowls at Ben-Shemen it seemed probable that these acted as carriers and an examination of apparently healthy native fowls proved this to be the case. Twenty-five apparently healthy native fowls from the Jerusalem market were examined and leucocytic inclusions indistinguishable from those found in the sick fowls at Ben-Shemen were found in three (i.e., 12 per cent.).

Macfie (1914) described an acute disease of fowls in Eket, Nigeria, generally fatal in two days ; the disease was characterised in the first stage by a tendency to stand stock-still with head and tail drooping, later the shoulders were hunched up, the head sunk, the tail feathers depressed, the feathers ruffled and the eyelids closed ; finally the birds were unable to stand and lay on the ground without attempting to move. Diarrhoea was a marked symptom.

Macfie found in the leucocytes of infected fowls chromatic granules and rings of a type not occurring in healthy fowls. The inclusions appeared in the blood of a healthy native fowl five days after inoculation with the blood of a diseased fowl. The inoculated native fowl, however, showed no ill-effects as a result of the inoculation. In 1915, Macfie found inclusions in the leucocytes of a sick turkey in Accra. Blood from the turkey proved infective to a cock which succumbed ten days after the infection. Inclusions were found in the leucocytes of the cock on the fourth day after the infection.

The leucocytic inclusions described and figured by Macfie appeared to the writer identical with those found at Ben-Shemen. Blood smears from a healthy native fowl and from a sick fowl in Ben-Shemen were sent to Dr. W. Scott Macfie, of the Liverpool School of Tropical Medicine. Dr. Macfie kindly examined the slides and agreed that they contain inclusions indistinguishable from those he found in sick fowls in Eket. He further added that the disease in which he found the leucocytic inclusions was common in fowls in the Gold Coast and Nigeria.

In view of the similarity of the leucocytic inclusions and the pathology of the disease of fowls as found in Nigeria and the disease as found in Palestine, we consider the two diseases to be identical. The fact that the disease as it occurs in Ben-Shemen is of longer duration than the disease described by Macfie in Nigeria, is no evidence against the identity of the two diseases, for in spirochaetosis of fowls there is also an acute form of the disease lasting three to five days and a chronic form lasting about a fortnight after the appearance of spirochaetes in the blood. In Palestine both the acute and chronic form of spirochaetosis of fowls is present, but the chronic form lasting about a fortnight is much the commoner.

The following experiments were carried out :

(1) 4.11.24, blood (2 c.c.) from the wing vein of a healthy native fowl, No. 3, was injected intramuscularly into a healthy native fowl, No. 11. Leucocytic inclusions were found daily in No. 3, since 26.10.24. No. 11 had been examined daily, from 2.11.24 to 4.11.24, and no leucocytic inclusions were found. The total number of leucocytes in No. 11, at the time of the injection, was 8,500 per cmm. Leucocytic inclusions were found in the blood of No. 11, on 9.11.24. The first forms to appear were minute granules lying in vacuoles ; two days later chromatic rings and other forms appeared. On the day the inclusions appeared a leucocytosis of 20,000 per cmm. was observed ; the leucocytosis persisted for several days. Leucocytic inclusions persisted in the blood till 5.12.24. The fowl appeared healthy throughout an observation period of two months.

This experiment was repeated on healthy native fowls, No. 6, No. 7, No. 12, No. 13, with similar results. Chromatic inclusions in the leucocytes appeared five to six days after the injection, the first appearance of the inclusions being accompanied by a leucocytosis in one case, No. 12, up to 34,000 per cmm. Of the five healthy native fowls thus infected, one, No. 7, died ten days after the injection but the others appeared none the worse for the infection.

(2) Blood (2 c.c.) from the wing vein of No. 12 was injected into two healthy native fowls, No. 4 and No. 5, on 13.11.24. No. 4 and No. 5 had been under observation since 2.11.24 and no chromatic inclusions were found in their leucocytes. Chromatic inclusions were found in No. 4 on 19.11.24, and in No. 5 on 20.11.24. Neither of the two injected birds were affected by the injection.

(3) Blood (4 c.c.) from No. 3 was defibrinated and filtered through a Berkfeld filter. The filtrate was injected intramuscularly, on 13.11.24, into healthy native fowls, No. 8 and No. 9. These had been under observation since 2.11.24 and their leucocytes appeared free from the above described chromatic inclusions ; inclusions appeared in the leucocytes of No. 8 on 19.11.24. The bird died on 30.11.24 and smears of the blood and organs showed numerous leucocytic inclusions. Inclusions appeared in the leucocytes of No. 9 on 18.11.24, but no pathological results were noted as a result of the injection. It appears that the minute granules lying in vacuoles are infective, for the other forms of inclusions are too large to pass through a Berkfeld filter.

(4) Blood (2 c.c.) from No. 4 was injected into two healthy Leghorn cocks, No. 26 and No. 27, on 19.12.24. The leucocyte count of No. 26, at the time of the

experiment, was 6,400 per cmm. Chromatic inclusions were found in the leucocytes on 24.12.24. A leucocytosis of 18,000 per cmm. was noticed on the previous day, 23.12.24. Till 26.12.24 the only forms of inclusions noted in the leucocytes were minute granules lying in vacuoles; on 28.12.24 the other forms appeared. The bird was noticed to be ill on 24.12.24. It refused food and stood perfectly still; the temperature rose to 110° F. On 30.12.24 diarrhoea was noticed, the stools being greenish and on microscopical examination being found to contain numerous fat globules. Death took place on 31.12.24. Post mortem: the liver was found to be soft and fatty. Blood smears and organ smears showed numerous chromatic inclusions in the leucocytes.

In No. 27, leucocytic inclusions appeared in small numbers on 25.12.24. The leucocyte count on 19.12.24, the day of the injection, was 8,510 per cmm., and it rose to 14,500 per cm. on 25.12.24. The temperature rose to 110° F. on 24.12.24. The bird appeared ill from 24.12.24 till 30.12.24, and then recovered. Chromatic inclusions were present in small numbers in the leucocytes till 18.1.25.

(5) Highly infected blood (1 c.c.) from No. 12 was injected intramuscularly into three pigeons on 13.11.24. Chromatic inclusions such as described above were never found in the leucocytes of the pigeons during an observation period of six weeks.

Observations on healthy native fowls whose leucocytes contained chromatic inclusions showed that the inclusions persisted in the blood during a period varying from one to seven weeks. During this period crises of leucocytosis lasting one to two days were noted at irregular intervals. The leucocyte count rose to 34,000 per cmm. in one case. In this connection it is interesting to note that a blood smear from a sick fowl which died at Ben-Shemen was sent by Mr. D. Ury to the laboratory and a diagnosis of leukaemia was established; chromatic inclusions were found in the leucocytes, but whether the inclusions were aetiologically related to the leukaemia or whether, as is more probable, the case was a mixed infection of leukaemia and the disease described by Macfie, it is impossible to say, as the author could not find any other cases of *Leukaemia gallinarum* in Ben-Shemen or in Jerusalem. It seems unlikely that the leucocytic inclusions are related to *Leukaemia gallinarum* since, according to Ellerman and Bang (1908), the latter disease has an incubation period of one to two months.

THE RELATIONSHIP OF THE LEUCOCYTIC INCLUSIONS TO THE DISEASE

The constancy with which the leucocytic inclusions are found in the disease and the fact that the inclusions appear regularly in the blood of inoculated fowls, led Macfie to conclude that the inclusions are true parasites belonging probably to the Chlamydozoa and are

causally related to the disease. The above observations support Macfie's view.

That the leucocytic inclusions are not products of cell degeneration is proved by the fact that in highly-infected cells, particularly in lymphocytes, they may be so numerous as to exceed in volume the nucleus of the host cell. Moreover, with Giemsa they stain more brightly than the nucleus of the host cell.

DISTRIBUTION OF THE DISEASE

Since the disease is common in Nigeria and the Gold Coast and is present in Palestine, it seems probable that it is also present throughout the whole of North-west Africa and throughout the whole of North Africa and Egypt, but has hitherto escaped attention owing to its clinical resemblance to spirochaetosis.

TREATMENT

Atoxyl by mouth and neo-salvarsan intramuscularly, did not cause the disappearance of the leucocytic inclusions in healthy native fowls or in sick fowls from Ben-Shemen, and produced no effect on the course of the disease in the latter.

Injections of Bismuth Sodium tartrate (to which fowls are remarkably tolerant, 0·8 gms. per kilo-body-weight producing no ill-effects) also proved useless. The above therapeutic tests suffice to differentiate the disease from spirochaetosis, for spirochaetosis of fowls in Palestine as elsewhere yields readily to treatment with atoxyl or neo-salvarsan and 0·03 gms. per kilo-body-weight of bismuth sodium tartrate was found to be sufficient to cure fowls of spirochaetosis in Jerusalem.

TRANSMISSION

Native fowls from the Jerusalem market and diseased fowls from Ben-Shemen were examined for ectoparasites ; *Mallophaga* sp. were observed and *Argas persicus* was found to be very common ; the experimental farm at Ben-Shemen was found to be heavily infested with *Argas persicus*. It was expected that *Argas persicus* would

prove to be the carrier and the following experiments were carried out :—

(6) Fifty specimens of *Argas persicus*, taken from the farm at Ben-Shemen, were macerated in 10 c.c. saline. After maceration the resulting brown fluid was injected intramuscularly into four native fowls. The injected fluid acted as a strong local irritant and also produced general toxic results and a marked leucocytosis. The fowls appeared depressed for several days after the injection and one died three days later. The other three recovered and none showed the typical chromatic inclusions in their leucocytes during an observation period of two weeks and none developed spirochaetosis.

(7) Three batches, each of ten specimens of *Argas persicus*, which had been kept in the laboratory several months without a feed, were allowed to bite but not to complete a feed on an infected fowl, No. 4, whose blood contained all the above described varieties of leucocytic inclusions ; the ticks were then allowed to bite three native fowls, five, twelve, and twenty days later. In no case did the chromatic inclusions appear in the leucocytes during an observation period of three weeks.

(8) A native fowl whose blood showed a natural infection of the above-described leucocytic inclusion was placed in a cage with nine other native fowls whose blood, at the time (24.10.24), appeared free from the inclusions. The birds were not allowed out of the cage. A month later all were examined and the leucocytic inclusions were found in six.

The above experiments are, however, not sufficient to exclude the probability of *Argas persicus* being the carrier of the disease. Even Experiment No. 8 cannot be regarded as conclusive, for although care was taken to exclude *Argas persicus*, yet this parasite is so common in Palestine that its absence from the cage for a whole month in Experiment No. 8 cannot be guaranteed.

I have to thank Mr. D. Ury, of Ben-Shemen, for supplying me with material ; Dr. A. Felix, Pathologist to the Rothschild Hospital, Jerusalem, for kindly allowing me the use of his laboratory ; and Dr. W. Scott Macfie, for kindly examining blood smears.

SUMMARY AND CONCLUSIONS

A disease of fowls in Palestine characterised by various forms of chromatic inclusions in the leucocytes is described.

The inclusions appear to be identical with the leucocytic inclusions described and figured by Macfie from Eket, Nigeria.

The disease in Palestine is considered to be the chronic form of the disease described by Macfie.

The disease can be transferred to healthy fowls by blood inoculation.

The inclusions appear five to six days after the inoculation of infected blood.

The inclusions are considered to be true parasites belonging to the Chlamydozoa.

Transmission experiments with *Argas persicus* were unsuccessful, but the experiments are not conclusive.

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